THE GONADOTROPIC EFFECTS OF REFRIGERATOR-PRESERVED ANTERIOR PITUITARY

by

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INTRODUCTION

It is generally accepted that in the case of implantation of the anterior pituitary into immature animals, precocious sexual maturity appears on the genital system of recipients. However, there is no evident proof as to whether its gonadotropic activity is achieved only by the action of the hormone which has been stored at the time of its implantation, or whether the cells which have survived for a certain period after implantation carry on their secretion, and the action of the hormone thus secreted is added to that of the hormone preserved in advance.

For the purpose of examining the above problem, I have carried out the following experiments.

The gonadotropic hormone of the anterior pituitary is said to be considerably unstable and easily broken down especially by heat, but if preserved below 4 degrees centigrade it becomes stable and hardly destroyed. Taking this fact into consideration, if the hypophysis is kept in a refrigerator in a form of an entire organ for a fixed duration, it can be assumed that the activity of gonadotropic hormone of the pituitary will not decrease rapidly. However the pituitary tissue shows some degeneration as a result of being preserved in the refrigerator.

In the present study heterogeneous implantation of the anterior pituitary which had been preserved in the refrigerator was carried out on immature animals and the gonadotropic effects were examined. At the same time, as a control, the same anterior pituitary was implanted on another group of animals in its fresh state and the gonadotropic effects were examined. Moreover the anterior pituitary preserved in the refrigerator which was ready for implantation was histologically examined.

MATERIALS AND METHODS

For this experiment, immature hybrid female mice, 20 to 26 days old, were used. The pituitaries to be used for implantation were obtained from cows. After having secured the pituitary covered with a capsule from the slaughter house, the disinfection of the pituitary was extracapsularly performed with a solution of 5 per cent iodine tincture and 2 per cent sodium thiosulfate alcohol, and then the

capasule was aseptically removed. The anterior lobe of the pituitary, having been separated from the posterior lobe, was immersed in a physiologic saline solution including penicillin for about 15 minutes after which the anterior pituitary was cut into halves. The left half which was to be used as a control was either implanted immediately in its fresh state or injected as a fresh emulsion. The right half was kept in an aseptic container and preserved in 0 to 2 degrees centigrade in a refrigerator and either implanted or injected as an emulsion. The quantity for implantation was fixed at 0.1 gram which was subdivided into pieces of about 10-20 milligrams and then implanted to an immature mouse subcutaneously. The recipient was killed 96 hours after the implantation and the uterus and ovaries were weighed. The latter were fixed in 10 per cent formalin, imbedded in paraffin and stained with hematoxylin-eosin after which the follicles and corpora lutea were histologically examined. Previous to the recipient being killed it was first examined whether the vaginal introitus was opened or not and how the characters of the vaginal smear were.

For histological examination the anterior pituitaries, whether they may be fresh or have been preserved in refrigerator for various periods, were first fixed in BOUIN'S fluid and then imbedded in paraffin and stained by GOMORI'S method and hematoxylin-eosin.

As the body weight of each mouse differed according to the circumstances and conditions of breeding, each recipient was selected by its age. The weight of a mouse about three weeks old was roughly from 6 to 8 grams. The vaginal introitus was always closed and no sexual cycle of vaginal smear was noticed. The weight of the uterus was 5-6 milligrams, and the ovary about 2 milligrams. The histological examination of the ovary revealed that it had no mature follicle but mostly primordial ones and medium- or small-size follicles, which consisted of several layers of granulosa cells. Corpus luteum could not be found. (Fig. 1).

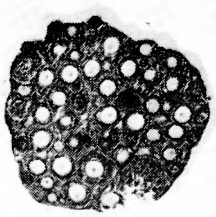


Fig. 1 : The ovary of untreated immature normal mouse. (Hematoxylin-Eosin stain ×40)

RESULTS

I. The Implantation of the Anterior Pituitary.

Each one of the cow's anterior pituitaries was cut into halves. The one half was preserved in a refrigerator for 5, 10, 20 and 30 days respectively. Then the sexual maturity of the mice which had received implantation of the pituitaries preserved in the refrigerator was compared with that of the mice which had received implantation of fresh pituitaries. With the use of one pituitary, an experimental group, consisting of 3 mice receiving implantation of the preserved half of the pituitary and 3 mice receiving the fresh half was established. All the mice belonging to one experimental group were of the same date of birth. For each

experiment, according to the period of days of preservation, three pituitaries or, namely, three experimental groups were used respectively.

1) Implantation of the Anterior Pituitary Preserved in the Refrigerator for 5 Days: In the item of the vaginal smear on the tables P stands for prooestrus, O for oestrus, M for metaoestrus, and D for dioestrus. The weights of the uteri and ovaries indicate the lowest and highest of the three mice. The numbers of mature follicles and corpora lutea are indicated by + which signifies from 1 to 4, ++ from 5 to 9, and +++ more than 10. Follicles that are not perfectly matured but in their growing stage are shown by the sign ÷.

Of the results obtained by implantation of the anterior pituitary preserved in a refrigerator for five days, as shown in Table 1, the opening of the vaginal introiti was noticed about 72 hours after the implantation in all cases and the

Table 1. Implantation of the Anterior Pituitary Preserved in the Refrigerator for 5 Days.

Group	W. P. g	Subgroup (Impl. Pit.)	Impl. Quantity g	No. of Mice	Opning of Vaginal Introitus	Vaginal Smear	Weight of Uteri mg	Weight of Ovaries mg	Histological Changes of Ovaries: Mature Follicles − ÷ + ++ +++	Histological Changes of Ovaries: Corpora lutea − + ++ +++
I	1.2	1) Fresh	0.1	3	+	O	40–50	4-7	2 1	2 1
		2) Preserved	0.1	3	+	O. M	28–40	4–6	1 1 1	2 1
II	1.3	1) Fresh	0.1	3	+	P. O	22–28	4–6	3	3
		2) Preserved	0.1	3	+	O. M	18–26	4–6	2 1	2 1
III	1.2	1) Fresh	0.1	3	+	O	25–30	4–7	3	1 2
		2) Preserved	0.1	3	+	O	25–42	4–8	3	2 1

W. P. : Weight of cow's anterior pituitary as a whole.
P : Prooestrus, O : Oestrus, M : Metaoestrus, D : Dioestrus.

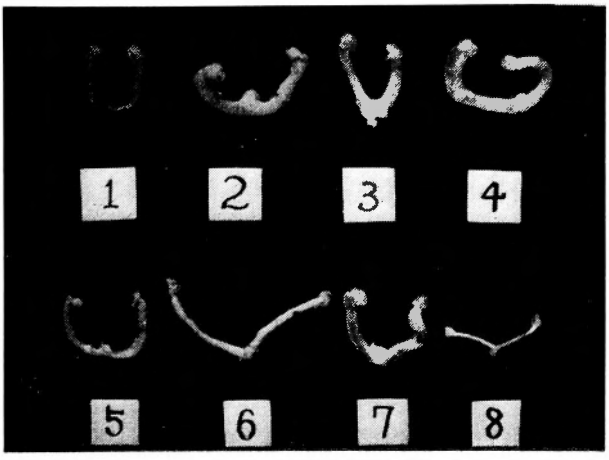


Fig. 2 : Uterus (96 hours after implantation) : 1. Uterus of an untreated immature mouse. (25 days old). 2. Uterus in the case of fresh pituitary implantation. 3. Uterus in the case of implantation of pituitary preserved in the refrigerator for 5 days. 4.—10 days. 5.—20 days. 6. 7.—30 days. 8. Uterus in the case of implantation of pituitary immersed in a large amount of physiologic saline solution and preserved in the refrigerator for 5 days.

vaginal smear brought about a sexual cycle as shown also in the Table 1. The weights of the uteri and ovaries increased both in the subgroups of mice implanted with fresh pituitaris and in those implanted with pituitaries preserved in a refrigerator, and the uteri were filled with secretion. (Fig. 2). In the first, second and third groups respectively, mature follicles were more or less found in each subgroup. Most of the mature follicles were hemorrhagic and large cystic. Corpora lutea were found in the ovaries of all groups except in the fresh-pituitary subgroup of the second group. (Fig. 3 and 4). However, it was quite difficult to ascertain a distinct difference between the two subgroups in the weights of the uteri and ovaries, in the number of mature follicles and corpora lutea and etc. because, as shown in the Table 1, the values in the preserved-pituitary subgroup were sometimes more and sometimes less as compared with those of the fresh-pituitary

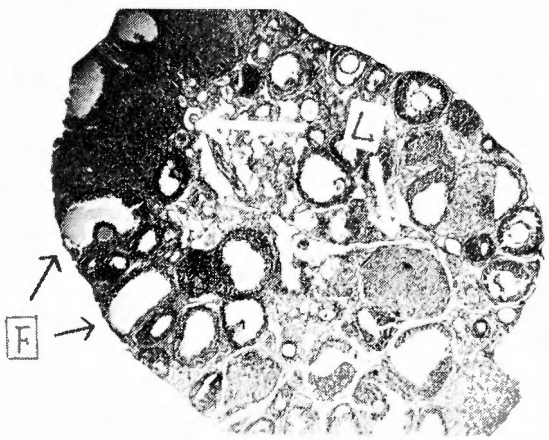


Fig. 3 . The ovary in the case of fresh pituitary implantation(96 hours after implantation) F : mature follicle L : corpus luteum There are many cystic follicles and corpora lutea. (Hematoxylin-Eosin stain ×40)

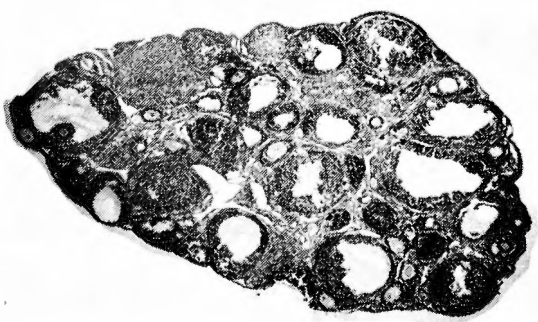


Fig. 4 : The ovary in the case of implantation of the pituitary preserved in the refrigerator for 5 days. There are many cystic follicles and few corpora lutea. (Hematoxylin-Eosin stain ×40)

subgroup.

2) Implantation of the Anterior Pituitary Preserved in the Refrigerator for 10 Days: As shown in Table 2, the vaginal smear and the weights of the uteri and ovaries showed the same responses as those obtained in the previous experiment. The mature follicles were seen on the ovaries in all tests. The corpora lutea could not be noticed in either subgroup of the second group but could be seen in the first and third groups. Consequently, the response of the genital system throughout all the test groups can be said to show no distinct difference between the two subgroups.

3) Implantation of the Anterior Pituitary Preserved in the Refrigerator for 20 Days: In the first and second groups, corpora lutea could not be found in either subgroup but other phenomena of maturity were noticed. No distinct difference in gonadotropic effects could be noticed between the two subgroups. (Table 3).

4) Implantation of the Anterior Pituitary Preserved in the Refrigerator for 30 Days: As shown in Table 4, the genital system of both subgroups brought about a remarkable change. Especially, mature follicles and corpora lutea in the ovaries in both subgroups of all groups indicated either +, ++ or +++. Also, in these groups, there was no distinct difference in gonadotropic effects between the

Table 2. Implantation of the Anterior Pituitary Preserved in the Refrigerator for 10 Days.

Group	W. P. g	Subgroup (Impl. Pit.)	Impl Quantity g	No. of Mice	Opning of Vaginal Introitus	Vaginal Smear	Weight of Uteri mg	Weight of Ovaries mg	Histological Changes of Oraries	
									Mature Follicles	Corpora lutea
									−÷+++	−+++
I	1.2	1) Fresh	0.1	3	+	O	40–75	5–10	2 1	3
		2) Preserved	0.1	3	+	O. M	25–55	4–7	1 2	1 2
II	1.1	1) Fresh	0.1	3	+	O. M	22–28	4–5	1 2	3
		2) Preserved	0.1	3	+	M	18–36	4–6	3	3
III	1.4	1) Fresh	0.1	3	+	O	20–45	6–8	3	1 2
		2) Preserved	0.1	3	+	O. M	22–35	6–10	2 1	2 1

Table 3. Implantation of the Anterior Pituitary Preserved in the Refrigerator for 20 Days.

Group	W. P. g	Subgroup (Impl. Pit.)	Impl. Quantity g	No. of Mice	Opning of Vaginal Introitus	Vaginal Smear	Weight of Uteri mg	Weight of Ovaries mg	Histological Changes of Ovaries	
									Mature Follicles	Corpora lutea
									−÷+++	−+++
I	1.2	1) Fresh	0.1	3	+	O. M	18–25	4–6	3	3
		2) Preserved	0.1	3	+	O. M	22–28	4–7	1 2	3
II	1.0	1) Fresh	0.1	3	+	O	30–35	6–10	3	3
		2) Preserved	0.1	3	+	O	28–40	5–10	1 2	3
III	1.0	1) Fresh	0.1	3	+	O. M	35–68	6–12	2 1	1 2
		2) Preserved	0.1	3	+	O	24–50	6–8	1 2	3

Table 4. Implantation of the Anterior Pituitary Preserved in the Refrigerator for 30 Days.

Group	W. P. g	Subgroup (Impl .Pit.)	Impl. Quantity g	No. of Mice	Opning of Vaginal Introitus	Vaginal Smear	Weight of Uteri mg	Weight of Ovaries mg	Histological Changes of Ovaries: Mature Follicles −÷+++	Histological Changes of Ovaries: Corpora lutea −+++
I	1.2	1) Fresh	0.1	3	+	O. M	35–62	7–14	2 1	3
		2) Preserved	0.1	3	+	O. M	30–42	5–7	3	3
II	1.0	1) Fresh	0.1	3	+	O. M	26–38	4–6	3	3
		2) Preserved	0.1	3	+	O. M	45–65	5–6	2 1	1 1 1
III	1.2	1) Fresh	0.1	3	+	O	22–52	5–6	2 1	2 1
		2) Preserved	0.1	3	+	O. M	40–54	6–10	1 2	2 1

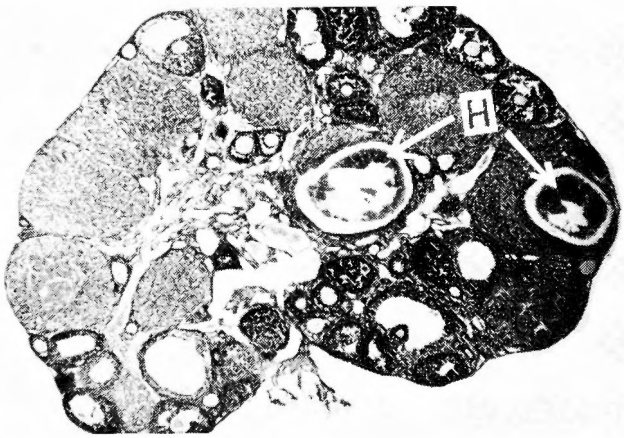


Fig. 5 · The ovary in the case of implantation of the pituitary preserved in the refrigerator for 30 days. H : bleeding in the corpus luteum. (Hematoxylin-Eosin stain ×40)

two subgroups. (Fig. 5).

5) Implantation of Smaller Quantities of the Anterior Pituitary Preserved in the Refrigerator: The experiments mentioned above were all carried out by implanting 0.1 gram of anterior pituitary into the recipients. If a quantity of less than 0.1 gram were implanted, it might be possible that the difference between the implantation of fresh pituitary and that of preserved pituitary may become distinct. Therefore the amount of each implanted pituitary was graded into 5, 10, 25 and 50 milligrams, and the anterior pituitary used for implantation was preserved in the refrigerator for 10 days or 30 days. Experimental methods were the same as previously mentioned.

The results of these experiments are shown in Tables 5 and 6. It was found that the grade of the precocious sexual maturity of the genital system was in proportion to the quantity of implantation. The less quantity of implantation was used, the less the gonadotropic effects. However in comparing the gonadotropic effects of the two subgroups, no distinct variation could be noticed in them throughout the entire experiments.

Table 5. Implantation of Smaller Quantities of the Anterior Pituitary Preserved in the Refrigerator for 10 Days.

Group	W. P. g	Subgroup (Impl. Pit.)	Impl Quantity mg	No. of Mice	Opning of Vaginal Introitus −	Opning of Vaginal Introitus +	Vaginal Smear	Weight of Uteri mg	Weight of Ovaries mg	Histological Changes of Ovaries: Mature Follicles −÷+++	Histological Changes of Ovaries: Corpora lutea −+++
I	1.5	1) Fresh	5	3	3		−	8–15	2–4	2 1	3
			10	3	3		−	12–24	4–5	2 1	3
			25	3	1	2	− O	16–38	4–6	3	2 1
			50	3		3	O. M	35–46	5–7	2 1	2 1
		2) Preserved	5	3	3		−	8	2–3	2 1	3
			10	3	2	1	−	10–18	2–3	3	3
			25	3	1	2	− M	10–35	2–5	3	3
			50	3		3	O. M	25–75	4–6	3	3
II	1.2	1) Fresh	5	3	3		−	8–12	2–4	2 1	3
			10	3	2	1	−	10–25	2–5	1 2	3
			25	3	1	2	− P. O	15–20	3–4	1 2	3
			50	3		3	O. M	25–35	5–6	3	3
		2) Preserved	5	3	3		−	7–12	2–3	3	3
			10	3	2	1	−	10–22	2–3	2 1	3
			25	3	2	1	− P	10–20	2–4	3	3
			50	3		3	O. M	16–32	3–5	2 1	1 1 1
III	1.0	1) Fresh	5	3	3		−	8–13	2–3	2 1	3
			10	3	3		−	6–10	2–3	2 1	3
			25	3	3		−	8–18	3	2 1	3
			50	3	1	2	− M	30–48	5–6	2 1	3
		2) Preserved	5	3	3		−	6–8	2–3	2 1	3
			10	3	3		−	10–12	2–4	1 2	3
			25	3	3		−	10–15	3–5	1 2	3
			50	3	1	2	− O	15–36	4–6	2 1	3

II. Implantation of the Anterior Pituitary Immersed in a Large Amount of Physiologic Saline Solution and Preserved in the Refrigerator.

According to the previous experiments, it seemed likely that the gonadotropic effects of implantation of the anterior pituitary preserved in a refrigerator was entirely due to the absorption of the stored hormone. Therefore, the effects of implantation of the pituitary immersed in a large quantity of saline solution and preserved in a refrigerator were examined, which must have lost the storage of the hormone by the release of the hormone into the physiologic saline solution.

As a control, one half of the pituitary was immediately implanted in its fresh state in the same manner as previously described. The remaining half was subdivided to about 10-20 milligrams and kept in about 300 cc of physiologic saline solution. After having been preserved in a refrigerator for 1, 3 and 5 days, they were taken out and implanted. The amount of each implantation was 0.1 gram.

The results obtained from this experiment, as shown in Tables 7 and 8, indicated a remarkable sexual maturity of the control mice, but no marked or visible change in the mice, which had received the implantation of the pituitary immersed in a large amount of saline solution and preserved in a refrigerator. In other words, all the mice of the control subgroups showed the opening of the vaginal introitus and the sexual cycle, and a remarkable increase ofweights of the

Table 6. Implantation of Smaller Quantities of the Anterior Pituitary Preserved in the Refrigeretor for 30 Days.

Group	W. P. g	Subgroup (Impl. Pit.)	Impl. Quantity	No. of Mice	Opning of Vaginal Introitus −	Opning of Vaginal Introitus +	Vaginal Smear	Weight of Uteri mg	Weight of Ovaries mg	Histological Changes of Ovaries: Mature Follicles − ± + ++ +++	Histological Changes of Ovaries: Corpora lutea − + ++ +++
I	1.4	1) Fresh	5	3	3		—	6-15	3-4	2 1	3
			10	3	2	1	— O	6-18	2-4	1 1 1	3
			25	3		3	O. M	30-34	4-5	1 2	3
			50	3		3	O	45-75	4-8	1 2	3
		2) Preserved	5	3	3		—	6-7	2-3	3	3
			10	3	2	1	— P	8-15	3-4	1 2	3
			25	3		3	O. M	12-20	3-7	2 1	3
			50	3		3	O. M	25-45	4-6	1 2	3
II	1.2	1) Fresh	5	3	3		—	6-12	3	3	3
			10	3	2	1	— P	10-30	2-3	1 1 1	3
			25	3		3	O	25-40	3-4	3	3
			50	3		3	O	25-40	4-6	3	3
		2) Preserved	5	3	3		—	7-12	2-3	2 1	3
			10	3	3		—	5-7	2-3	2 1	3
			25	3	3		—	10-15	2-3	2 1	3
			0	3		3	O. M	22-35	3-5	3	3
III	1.2	1) Fresh	5	3	3		—	6-10	2	2 1	3
			10	3	3		—	6-8	2	2 1	3
			25	3	3		—	8-10	2-3	3	3
			50	3		3	— M	18-40	3-4	1 2	3
		2) Preserved	5	3	3		—	8-10	2-3	1 2	3
			10	3	3		—	8-18	2-3	3	3
			25	3	1	2	— O	25-32	3-5	1 2	3
			50	3		3	O. M	42-50	4-5	1 2	3

uteri and ovaries, but many of the experimental subgroups which had undergone the implantation of the anterior pituitary saline-immersed and preserved in a refrigerator for 1 and 3 days, did not show the opening of the vaginal introitus, the sexual cycle, and the remarkable increase of the weights of their uteri and ovaries. As regards ovarial changes, similarly, almost all the mice in these subgroups showed a weaker response than those in the control subgroups. In the case of implantation of pituitary immersed in a saline solution and preserved in a refrigerator for about 5 days, the difference between the two subgroups was more remarkable. (Figs. 2 and 6).

III. Histological Changes of the

Fig. 6 : The ovary in the case of implantation of the pituitary immersed in a large amount of physiologic saline solution and preserved in the refrigerator for 5 days. There is neither corpus leteum nor cystic follicle, but there are many follicles which are growing or primordial follicles. (Hematoxylin-Eosin stain ×40)

Table 7. Implantation of the Pituitary Immersed in a Large Amount of Physiologic Saline Solution and Preserved in the Refrigerator for 1 or 3 Days.

Group	W. P. g	Subgroup (Impl. Pit.)	Impl. Quantity g	No. of Mice	Opning of Vaginal Int-roitus −	Opning of Vaginal Int-roitus +	Vaginal Smear	Weight of Uteri mg	Weight of Ovaries mg	Histological Changes of Ovaries: Mature Follicles −÷+++	Histological Changes of Ovaries: Corpora lutea −+++
I	1.3	1) Fresh	0.1	3		3	O.M	32–62	4–6	3	3
		2) 1Day-Preserved Saline-Immersed	0.1	3	2	1	− O	10–35	2–4	1 2	3
		3) 3Days-Preserved Saline-Immersed	0.1	3	2	1	− O	8–32	2–4	2 1	3
II	1.2	1) Fresh	0.1	3		3	O	36–45	6–12	2 1	3
		2) 1Day-Preserved Saline-Immersed	0.1	3	1	2	− M	22–40	3–6	1 2	3
		3) 3Days-Presrved Saline-Immersed	0.1	3	3		−	10–15	2–3	2 1	3
III	1.4	1) Faresh	0.1	3		3	O	34–65	6–15	2 1	3
		2) 1Day-Preserved Saline-Immersed	0.1	3		3	O	20–26	3–4	3	3
		3) 3Days-Preserved Saline-Immersed	0.1	3	1	2	− O	12–30	3–6	1 2	3

Table 8. Implantation of the Pituitary Immersed in a Large Amount of Physiolgic Saline Solution and Preserved in the Refigeratorfor 5 Days.

Group	W. P. g	Subgroup (Impl. Pit.)	Impl. Quantity g	No. of Mice	Opning of Vaginal Int-roitus −	Opning of Vaginal Int-roitus +	Vaginal Smear	Weight of Uteri mg	Wegiht of Ovaries mg	Histolgical Changes of Ovaries: Mature Follicles −÷+++	Histolgical Changes of Ovaries: Corpora lutea −+++
I	1.6	1) Fresh	0.1	3		3	O. M	22–38	4–5	1 2	3
		2) Preserved Saline-Immersed	0.1	3	3		−	6–15	3	2 1	3
II	1.1	1) Fresh	0.1	3		3	O. M	25–42	4–6	1 2	1 1 1
		2) Preserved Saline-Immersed	0.1	3	3		−	8–13	2–3	2 1	3
III	1.0	1) Fresh	0.1	3		3	O. M	42–68	8–12	1 2	1 1 1
		2) Preserved Saline-Immersed	0.1	3	1	2	− O	12–20	3–4	1 2	2 1

Cow's Anterior Pituitary Preserved in the Refrigerator.

Gomori's stain method was applied. The results obtained were as follows: Acidophilic granules revealed as orange, basophilic granules as green or bluish-green, cytoplasm of chromophobe cells unstained or pale grayish-green.

1) Fresh Anterior Pituitary of a Cow: This contained a great amount of acidophilic cells and chromophobe cells, and a small amount of basophilic cells. Most of the basophilic cells concentrated in the peripheral part close to the capsule

and at the center. Most of the basophilic granules were green and bluish-green. Acidophilic cells dominated the entire area. (Fig. 7).

2) The Anterior Pituitary of a Cow Preserved in the Refrigerator for 5 Days: In some of the acidophilic cells, especially those in the outer part nuclei were slightly degenerated, but the cytoplasm and granules of such cells showed scarcely any change. Basophilic cells showed similar but stronger changes. However, the stainability of the granules in these cells was well preserved. Generally speaking, except for the outer part, no remarkable change was found in these pituitaries, as compared with the fresh ones. (Fig. 10).

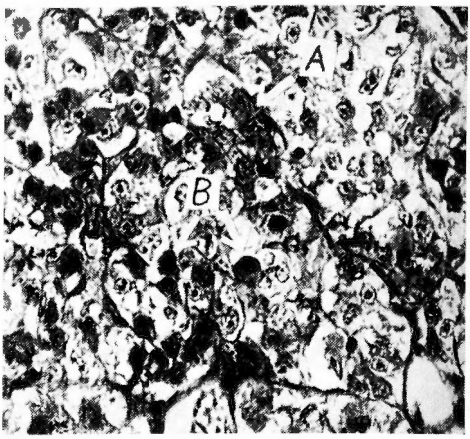
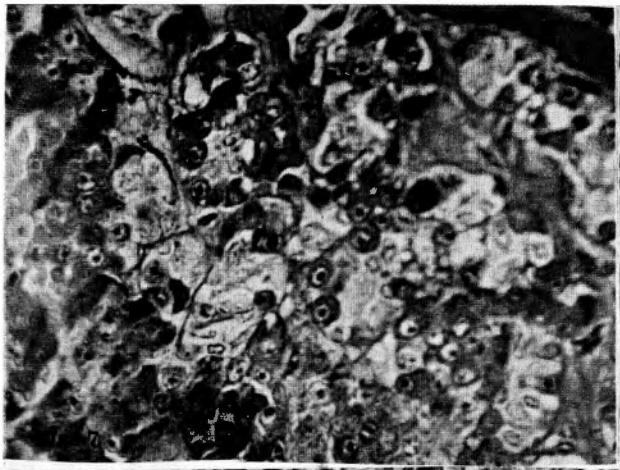


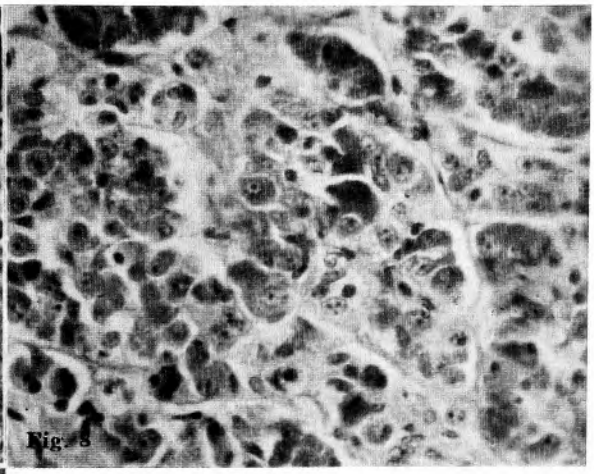
Fig. 10: Central area of anterior pituitary preserved in the refrigerator for 5 days. A: Acidophilic cell B: Basophilic cell (GOMORI's stain ×400)

3) The Anterior Pituitary of a Cow Preserved in the Refrigerator for 10 Days: The degeneration of the outer part of these pituitaries was more prominent than that of the pituitaries which were preserved in the refrigerator for 5 days. The basophilic cells, both nuclei and cytoplasms, were considerably atrophic but it was still possible for the granules to be stained. The deterioration of the stainability of the basophilic granules was seen close to the circumference. The changes of the acidophilic cells were less than those of the basophilic cells and there were not a few acidophilic cells that showed no remarkable degeneration. The acidophilic cytoplasm became somewhat shrunken, occasionally homogeneous and deep stained. (Fig. 8).

4) The Anterior Pituitary of a Cow Preserved in the Refrigerator for 20 Days: Degeneration of the outer part became much more prominent and extensive. In this area, three kinds of cells could not be differentiated from each other. However, at the center they showed only slight changes. In the basophilic cells, pyknotic nuclei increased in number while not pyknotic ones still remained. The cytoplasm of these cells seemed to be more or less atrophic. The granules of the cells in the outer part were difficult to be stained and the cytoplasm appeared to be homogeneous. Therefore, it was difficult to distinguish them from the chromophobe cells. The granules near the center indicated a bluish-green color similar to that of normal cells. Most of the acidophilic cells were also atrophic. The cytoplasm became reduced in size but the stainability of these granules remained unchanged. The changes of the chromophobe cells were somewhat less than those of chromophilic cells. (Fig. 11).

5) The Anterior Pituitary of a Cow Preserved in the Refrigerator for 30 Days. Macroscopically, the pituitary showed a remarkably dehydrated and atrophic appearance. Histologically the degeneration of the tissue became stronger: the





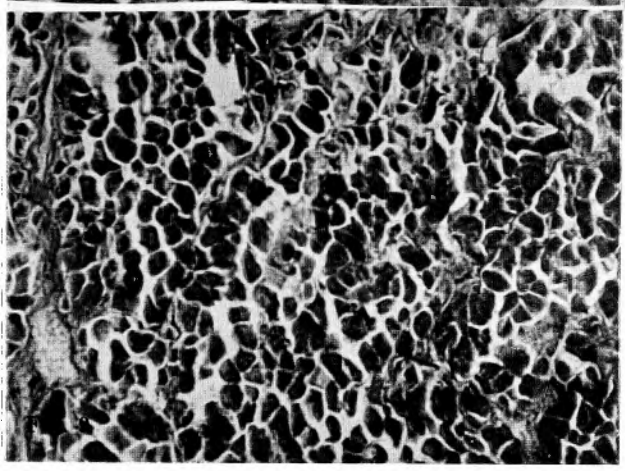


Fig. 7 : Fresh anterior pituitary. Acidophilic granules : orange. Basophilic granules : green (GOMORI's stain ×400)

Fig. 8 : Anterior pituitary preserved in refrigerator for 10 days (GOMORI's stain × 100)

Fig. 9 : Central area of anterior pituitary preserved in the refrigerator for 30 days. The nuclei are pyknotic, and cytoplasms are atrophic (GOMORI's stain ×400)

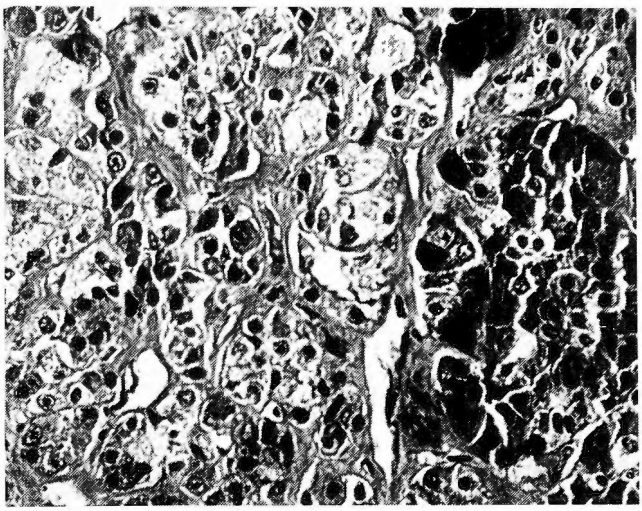


Fig. 11 : Anterior pituitary preserved in the refrigerator for 20 days. Remarkable degeneration is found in outer part of the pituitary (right), slight changes in central part (left). The nuclei are pyknotic and cytoplasms are atrophic. (GOMORI's stain ×400)

nuclei and cytoplasms of three kinds of cells were undergoing intense degeneration and the acini became smaller. The changes taking place in the outer part were especially prominent. In this area, it is, of course, impossible to differentiate the three kinds of cells from each other. As the cytoplasms of the basophilic cells were poorly stained, it was impossible to differentiate them from chromophobe cells. Moreover, acidophilic cells arranged themselves into a dumpling-like shape, mingling closely en masse, and were stained in a nasty color. Therefore, it was difficult to differentiate each nucleus from the cytoplasm. Even in the central part of these pituitaries, all the nuclei of the basophilic and acidophilic cells became

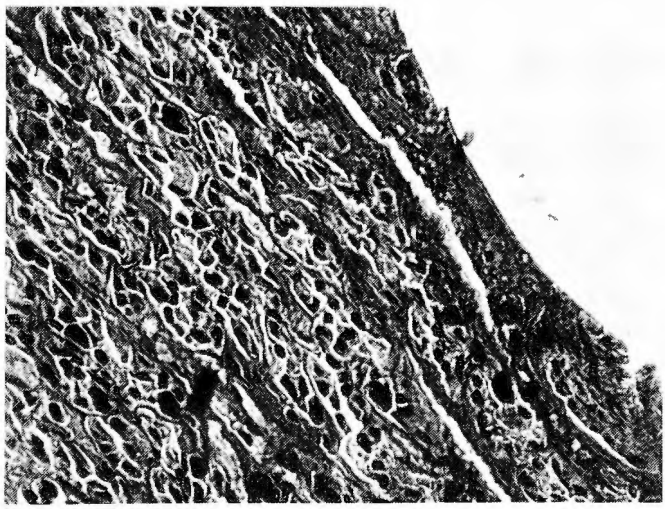


Fig. 12 : Outer part of anterior pituitary preserved in the refrigerator for 30 days. Remarkable degeneration is observed. (GOMORI's stain ×400)

pyknotic. The cytoplasm of these cells especially of acidophilic cells were reduced in volume and became square in form. (Figs. 9 and 12).

6) The Anterior Pituitary of a Cow Immersed in a Large Amount of Physiologic Saline Solution as Small Fragments and Preserved in the Refrigerator: The nuclei of the cells in the pituitaries saline-immersed and preserved in a refrigerator for 1 or 3 days did not show any remarkable change but the granules could not be successfully stained. Pituitaries preserved for 5 days showed a marked change in their outer parts. Pyknotic nuclei increased in number and the granules of the chromophilic cells were reduced and their staining was exceedingly worse than that in pituitaries which had been preserved in the refrigerator for 5 days without immersing them in physiologic saline solution. (Fig. 13).

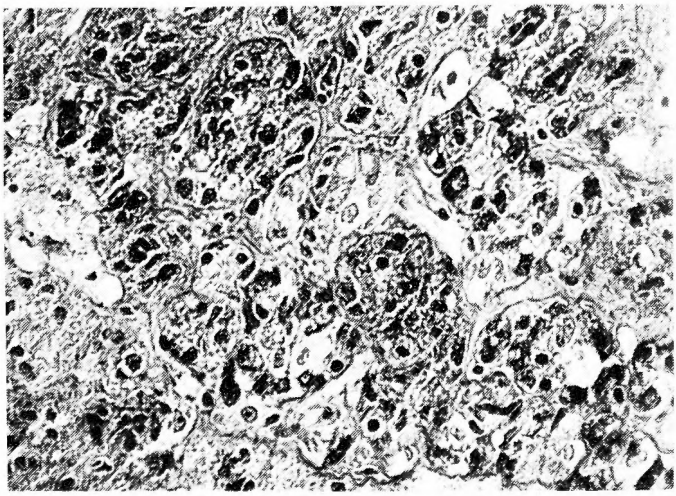


Fig. 13 : Anterior pituitary immersed in physiologic saline solution and preserved in the refrigerator for 5 days. The decrease in granules is observed (GOMORI's stain ×400) cf. Fig. 10

IV. The Injection of the Pituitary Emulsion Preserved in the Refrigerator for 30 Days.

Considering from the fact mentioned above that pituitaries preserved in the refrigerator degenerate commencing from their circumferences, it can be assumed that if they are kept in the refrigerator in form of an emulsion, the anterior pituitary tissues on the whole would soon die. In order to examine the gonadotropic effects of preserved emulsions, the following experiments were undertaken.

1) The emulsion was obtained by diluting a homogenized anterior pituitary about ten times (1.2 to 1.5 grams) with physiologic saline solution, to which a 50,000 unit penicillin was added. Half of the emulsion was preserved in the refrigerator at 0 to 2 degrees centigrade for 30 days. The remaining half was used as fresh emulsion for the control. The amount of 0.3 cc of this fresh emulsion was immediately injected into the subcutaneous tissue of immature female mice, and the rest was perserved in 0 to 2 degrees centigrade in the refrigerator and thereafter injected into mice at the rate of twice a day for a period of four days. The

total number of injections administered to one mouse was seven and the amount was 0.3 cc for each injection. These mice were killed 24 hours after the last injection (96 hours after the first injection), and the precocious sexual maturity of their genital systems were examined with the same method as explained in the implantation experiment. On the other hand, the remaining half of the emulsion preserved in the refrigerator for 30 days was also used for injection, the method being the same as previously mentioned, and their gonadotropic effects were compared with those of fresh emulsion.

Table 9. Injection of the Pituitary Emulsion Preserved in the Refrigerator for 30 Days. (1)

Group	W. P. g	Subgroup (Inj. Emul.)	No. of Mice	Opning of Vaginal Introitus −	Opning of Vaginal Introitus +	Vaginal Smear	Weight of Uteri mg	Weight of Ovaries mg	Histolgical Changes of Ovaries: Mature Follicles −÷+++	Histolgical Changes of Ovaries: Corpora lutea −+++
I	1.2	1) Fresh	3	2	1	− O	15–32	3–5	1 2	3
		2) Preserved	3	3		−	18–30	3–4	3	3
II	1.5	1) Fresh	3	2	1	− O	15–28	3–5	1 2	2 1
		2) Preserved	3	1	2	− M	18–28	4	3	2 1
III	1.5	1) Fresh	3	2	1	− M	12–26	3–4	1 2	2 1
		2) Preserved	3	2	1	− M	14–24	4–5	2 1	3

The results are shown in Table 9. In both subgroups receiving fresh and preserved emulsion injections gonadotropic effects on the genital system were less prominent than those obtained through the implantation method. Namely, in some of the recipients, the injection did not provoke the opening of the vaginal introitus and in some others, it only brought about a slight increase in the weights of the uteri and ovaries. There was no distinct difference in the gonadotropic effects between the fresh emulsion and the preserved one.

2) The Histological Changes of the Emulsion: When the sediments of the fresh emulsion were examined histologically (hematoxylin-eosin stain), it was found that some of the nuclei remained unchanged or were incompletely destroyed while others were completely destroyed. Cytoplasms were mostly disintegrated and only in few cells granules were recognized. (Fig. 14). In the emulsion of pituitaries preserved in the refrigerator for 30 days, all nuclei became strongly degenerated and the remains of the disintegrated nuclei were also visible. (Fig. 15). The granules were hardly visible. In short, the histological changes of the pituitary cells in this emulsion were more pronounced than those of the pituitary preserved en masse in the refrigerator for 30 days.

3) The histological changes of the sediments, as mentioned above, showed a remarkable difference between the fresh emulsion and the refrigerator-preserved emulsion. Now the emulsion was separated into two components; the fluid portion and the sediments and the gonadotropic effects of each component were examined separately.

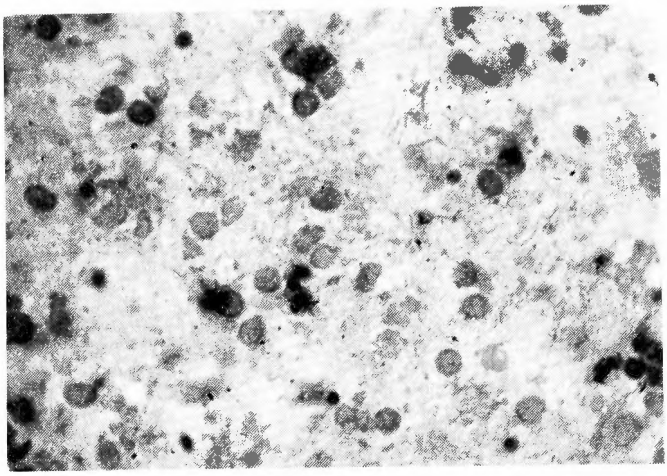


Fig. 14 : Fresh anterior pituitary emulsion. (Hematoxylin-Eosin stain ×400)

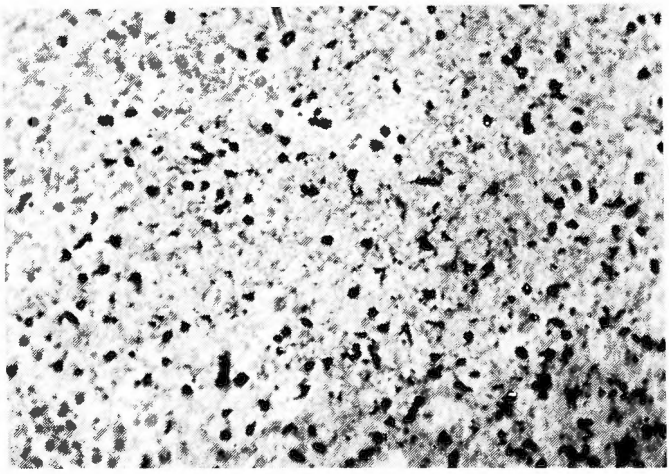


Fig. 15 : Pituitary emulsion preserved in the refrigerator for 30 days. All anterior pituitary cells are strongly disintegrated and degenerated, but faint granules still remain in some cells. (Hematoxylin-Eosin stain ×400)

In the same way as in the previous experiment, an emulsion diluted 10 times (original emlsion) was divided into two parts: the one half was preserved in the refrigerator for 30 days and the other half, in the state of a fresh emulsion, was separated by centrifugal sedimentation into the fluid portion and the sediments. After removing the fluid portion (initial fluid component), the sediments were dispersed in a physiologic saline solution, well stirred and then centrifuged again. After this renewed centrifugal sedimentation, the fluid portion was discarded. Then a physiologic saline solution was newly added to the sediments in an amount equivalent to the initial fluid component.

The final emulsion thus made (sediment component) and the initial fluid component were injected to two immature female mice separately by the same method as previously mentioned (0.3 cc for each injection, total number of injections

being 7). From the other half of the original emulsion preserved in the refrigerator for 30 days, two test materials for injection, i. e. the initial fluid component and the sediment component were made by the same method as the above, and tested for the gonadotropic effects in comparison with those of the similar two test materials from the original fresh pituitary emulsion.

Table 10. Injection of the Two Separated Components of the Pituitary Emulsion Preserved in the Refrigerator for 30 Days. (2)

Group	W. P. g	Subgroup (Inj. Emul.)		No. of Mice	Opning of Vaginal Introitus −	Opning of Vaginal Introitus +	Vaginal Smear	Weight of Uteri mg	Weight of Ovaries mg	Histolgical Changes of Ovaries: Mature Follicles − ÷ + ++ +++	Histolgical Changes of Ovaries: Corpora lutea − + ++ +++
I	1.2	1) Fresh	a) Fluid	2		2	O. M	18-25	3-5	1 1	2
			b) Sediments	2	1	1	− M	15-26	4	2	1 1
		2) Preserved	a) Fluid Portion	2	1	1	− M	12-24	4-5	1 1	2
			b) Sediments	2	2		−	8-12	2-4	1 1	2
II	1.3	1) Fresh	a) Fluid	2	1	1	− O	15-24	3-5	2	2
			b) Sediments	2		2	M	8-18	4	2	2
		2) Preserved	a) Fluid Portion	2	2		−	18-26	3-4	2	2
			b) Sediments	2	2		−	6-8	2-3	2	2
III	1.3	1) Freh	a) Fluid portion	2	1	1	− O	20-26	4-5	2	2
			b) Sediments	2		2	O. M	16-24	4-5	1 1	2
		2) Preserved	a) Fluid portion	2		2	O. M	18-28	4-5	2	1 1
			b) Sediments	2	2		−	8	2-3	1 1	2

The results are shown in Table 10. In the animals injected with the initial fluid component, the gonadotropic effects did not show any marked difference between the fresh and preserved emulsion. However, of the animal groups with injection of the sediment component, three subgroups receiving the sediments of the fresh emulsion showed some gonadotropic effects whereas the other three subgroups receiving the sediments of the preserved emulsion showed no gonadotropic effect whatsoever. In other words, the opening of the vaginal introitus and the sexual cycle never appeared and the weights of the uteri an ovaries were almost the same as normal. Mature follicles were hardly found, but primordial follicles and/or growing follicles were found. Moreover, there were no corpora lutea.

DISCUSSION

In summarizing the results obtained in the above experiments, the pituitaries preserved in the refrigerator for 5, 10, 20 and 30 days showed stronger degeneration in proportion to the number of the days of preservation, while the gonadotropic effects in these different test groups were about the same in grade. Therefore, in the case of implantation of such degenerated anterior pituitaries in immature mice, it is impossible to consider that the cells of these pituitaries would continue to secrete a hormone and thereby bring about precocious sexual maturity. However, the implantation of even such strongly degenerated pituitaries resulted in a remarkable precocious sexual maturity. Therefore, the gonadotropic effect of implants

of the pituitary preserved in the refrigerator seemed to result from the hormone which had been stored within the implanted tissue at the time of the implantation. In the case of emulsion preserved in the refrigerator the degenerative changes of the cells were quite remarkable, but no difference in the gonadotropic effect was observed between the preserved and fresh emulsion. This fact shows that even if the pituitary is preserved at 0-2 degree centigrade for 30 days, the effects of the gonadotropic hormone are hardly reduced.

Li reported that in isolating ICSH (Interstitial Cell Stimulating Hormone) chemically from acetone-dried sheep pituitary glands, all procedures should be carried out in a cold room below 4 degrees centigrade, and that the activity of ICSH remained unchanged as long as the pituitary glands were kept below 5 degree centigrade. The result of my experiments also proved this to be true.

The effect of gonadotropic hormone in the heterogeneous implantation of the fresh pituitary was usually temporary and the cells of the implanted pituitary were always short-lived. Accordingly, it is considered that the effect of the implantation results from the absorption of the hormone stored.

Honjo observed the histological changes of the heterogeneously implanted pituitary tissue. He reported that after implantation, revascularization did not occur in the implanted pituitary tissue, which fell into necrosis as a result of stoppage of blood supply, and also of tissue damage probably due to antibody formation.

According to the experiments carried out by Everett and Siperstein on the autoimplantation and homogeneous implantation of the fresh pituitaries, the hormone is not newly secreted from the implanted tissue immediately after implantation until the establishment of revascularization, but only the hormone stored in it is absorbed.

The results of my experiment showed that the gonadotropic effects of implantation of the heterogenous pituitary tissue which had been preserved in refrigerator were about the same as those of the pituitary tissue in the fresh state. From this fact, it is assumed that the effect of the implantation of the anterior pituitary preserved in the refrigerator is due to the absorption of the hormone which has been stored. Furthermore, the effects of this implantation are only temporary, because the revascularization is not established within the implanted tissue and the cells soon die. Accordingly, it is supposed that also the effects of gonadotropic hormone of heterogeneously implanted fresh pituitary are not due to the continued secretion of the hormone from the cells of the implanted tissue but to the absorption of stored hormone.

In the case of implantation of pituitaries immersed in a large quantity of physiologic saline solution and preserved in the refrigerator, the gonadotropic effect was distinctly less prominent than in the case of fresh pituitary implants, and the pituitaries thus saline-immersed and preserved in the refrigerator for five days were less effective than those likewise preserved for one to three days. As gonadotropic hormone of the anterior pituitary easily dissolves in water, it is supposed

that the decrease in effect seems to be due to the loss of the stored hormone by release into saline solution. In the case of injection of the fluid component of 10 times diluted pituitary emulsion, there was no distinct difference in the gonadotropic effect whether the fluid component was fresh or refrigerator-preserved, but in the case of injection of the sediments of the pituitary emulsion the preservation for a certain period of time caused a remarkable decrease in the gonadotropic effect. This fact seems also to be due to the release of effective hormone into the saline solution during the period of preservation in the refrigerator.

SUMMARY

PITUITARY IMPLANTATION

1) In the case of implantation into immature female mice of the pituitary of a cow, which had been preserved in 0 to 2 degrees centigrade in a refrigerator for a period of 5, 10, 20 and 30 days respectively, the gonadotropic effects did not have any relation with the days of cold storage but were about the same as those of fresh pituitary implants.

2) Also in an experiment where the quantity of implantation of the pituitary was reduced to various grades, no marked difference was found in the gonadotropic effect between the pituitary preserved in the refrigerator for 10 or 30 days and that in the fresh state.

3) The effect of the implants of small pituitary fragments which had been immersed in a large quantity of physiologic saline solution and preserved in the refrigerator, was less prominent than that of the fresh ones.

INJECTION OF PITUITARY EMULSION

4) The gonadotropic effect of the injection of pituitary emulsion preserved in the refrigerator for 30 days was almost equal to that of fresh emulsion.

5) The injection of the fluid component of the emulsion preserved in the refrigerator for 30 days produced nearly the same effects as those of the fresh one. Though the injection of the sediments of fresh emulsion brought about remarkable effects, no effect was obtained, on the contrary, by injecting the sediments preserved in the refrigerator.

HISTOLOGICAL CHANGES OF THE REFRIGERATOR-PRESERVED ANTERIOR PITUITARY

6) The anterior pituitary preserved in a refrigerator showed a strong degeneration in proportion to the days of cold preservation. Distinct changes predominated in the outer part of it, while at the center, it was possible to differentiate three kinds of cells even when the anterior pituitary was preserved in the refrigerator for 30 days. The change of basophilic cells took place quicker than that of acidophilic cells. The granules of cells in the pituitary fragments which had been immersed in a large quantity of physiologic saline solution and preserved in the refrigerator, could not so well be stained as those of the pituitary preserved en masse in the refrigerator. Sediments of pituitary emulsion preserved in the

refrigerator underwent an exceedingly remarkable degeneration.

Thanks are expressed to Dr. Naoki Kageyama for his kind suggestions during this study.

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和文抄録

冷蔵保存下垂体前葉の性腺刺戟ホルモン効果

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牛下垂体前葉を左右に切半して，半分は新鮮状態にて他の半分は種々の日数の間0-2℃の温度に冷蔵保存した後，夫々未成熟雌性マウスに移植，又は乳剤となして注射した．そして其の生殖器系に起る早期成熟現象を利用して，冷蔵保存下垂体前葉の性腺刺戟ホルモン効果と新鮮下垂体前葉のそれとを比較検討した．此の他に移植する下垂体の変化を Gomori 染色，Hematoxylin-Eosin 染色により組織学的に検索した．

其の知見を総括すると．

1）5, 10, 20, 30 日間夫々冷蔵保存した前葉を移植した場合，其の性腺刺戟ホルモンの効果は冷蔵日数と関係なく，対照の新鮮移植と同程度の効果を示した．

2）移植量を種々減量して比較した場合も同様に新鮮，冷蔵両者の間に著明な差異は認められなかつた．

3）大量の生理的食塩水に前葉を細切して浸漬しつゝ冷蔵したものの移植実験では，その効果は新鮮移植のそれよりも遙に少なかつた．此の事は下垂体前葉の性腺刺戟ホルモンは水溶性であるので，その有効成分が食塩水の方に移行した為であろうと考えられる．

4）30日間冷蔵保存下垂体前葉乳剤を注射した実験でも，その性腺刺戟効果は新鮮前葉乳剤のそれと殆んど差異がなかつた．

5）30日間冷蔵保存下垂体前葉乳剤の上層液注射による効果は新鮮乳剤上層液の場合と殆んど差異がなかつたが，沈渣注射では新鮮のものに於ては効果を示すのに反し，冷蔵のものでは殆んど効果を示さなかつた．此の事も3)と同様に有効成分は水溶液の方に移行した為であろうと考えさせる．

6）冷蔵保存下垂体前葉ではその冷蔵日数に比例して，退行変性は強くなつている．此の場合下垂体周辺部に変化が強い．併し中心部では30日間冷蔵した前葉でも3種の細胞の鑑別は可能である．細胞の変化は Basophil 細胞の方が Acidophil 細胞より早く起る様である．生理的食塩水に浸漬しつゝ冷蔵した前葉ではその顆粒の染色性は臓器の儘で冷蔵したものより低い．冷蔵保存乳剤の沈渣では変性が更に強度となてている．

7）以上の結果を併せ考えると冷蔵保存下垂体前葉移植の性腺刺戟ホルモン効果は，移植時移植片に既に貯えられていたホルモンが単に吸収されて起す作用であると考えられる．又新鮮異種下垂体前葉移植の場合の性腺刺戟効果も同様に単に貯えられていたホルモンの吸収によるのであろうと推定される．